

In the Claims:

Please cancel ~~claims 1-74¹~~ without prejudice or disclaimer of the subject matter thereof.

Applicants reserve the right to pursue the subject matter of these claims in continuing applications.

Please add the following new claims:

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- 75. A DNA molecule which encodes an RNA molecule comprising:
- (a) at least one *cis*-acting sequence element,
 - (b) a first open reading frame which encodes a non-cytopathic, temperature-sensitive RNA-dependent RNA polymerase, and
 - (c) at least one second nucleotide sequence selected from the group consisting of:
 - (i) a second open reading frame encoding a protein, or portion thereof,
- 02* wherein said second open reading frame is in a translatable format after one or more RNA-dependent RNA replication events;
- (ii) a sequence complementary to all or part of the second open reading frame of (i); and
 - (iii) a sequence encoding an untranslated RNA molecule, or complement thereof;
- wherein said second nucleotide sequence is operably linked to a promoter which is activated by said non-cytopathic, temperature-sensitive RNA-dependent RNA polymerase.

¹The Examiner indicates on page 1 of Paper No. 11 that claims 1-34, 38-70, and 74 are pending in the captioned application. Applicants point out, however, that prior to the cancellation of claims 1-74, claims 1-74 were pending in the captioned application.

76. The DNA molecule of claim 75, which comprises one second nucleotide sequence.

77. The DNA molecule of claim 75, wherein said second open reading frame is in a translatable format after one RNA-dependent RNA replication event.

78. The DNA molecule of claim 75, wherein said second open reading frame is in a translatable format after three RNA-dependent RNA replication events.

79. The DNA molecule of claim 75, wherein the RNA-dependent RNA polymerase is of viral origin.

80. The DNA molecule of claim ~~75~~ wherein the RNA-dependent RNA polymerase is of alphaviral origin.

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~~B²~~ } 81. The DNA molecule of claim ~~80~~, wherein the RNA-dependent RNA polymerase is derived from a Sindbis virus.

82. The DNA molecule of claim 75 which encodes an RNA-dependent RNA polymerase having replicase activity at 34°C which is at least five fold lower than the replicase activity exhibited at 29°C.

83. The DNA molecule of claim 75, wherein the second open reading frame encodes a cytokine, a lymphokine, a tumor necrosis factor, an interferon, a toxic protein, or a prodrug converting enzyme.

84. The DNA molecule of claim 75, wherein the second open reading frame encodes human erythropoietin or human β -interferon.

85. The DNA molecule of claim 75, wherein the second nucleotide sequence encodes an untranslated RNA molecule selected from the group consisting of an antisense RNA, a tRNA, a rRNA, or a ribozyme.

Q' 86. A method of making a recombinant host cell comprising introducing the DNA molecule of claim 75 into a host cell.

87. An *in vitro* cell culture comprising a recombinant host cell produced by the method of claim 86.

88. An *in vitro* cell culture comprising a recombinant host cell comprising the DNA molecule of claim 75.

89. The cell culture of claim 88, wherein some or all of the DNA molecule is stably maintained in said recombinant host cell.

90. An RNA molecule transcribed from the DNA molecule of claim 75.

91. An alphaviral particle containing the RNA molecule of claim 90.

92. An *in vitro* cell culture comprising a recombinant host cell comprising the RNA molecule of claim 90.

93. A method for producing a protein or an untranslated RNA molecule, said method comprising:

(a) introducing at least one DNA molecule of claim 75 into a host cell to produce a recombinant host cell;

(b) culturing the recombinant host cell under conditions suitable for expression of said protein or untranslated RNA molecule; and

(c) recovering said protein or untranslated RNA molecule;

wherein said protein or untranslated RNA molecule is encoded by said DNA molecule.

94. A method for producing a protein or an untranslated RNA molecule, said method comprising:

(a) introducing at least one RNA molecule of claim 90 into a host cell to produce a recombinant host cell;

(b) culturing the recombinant host cell under conditions suitable for expression of said protein or untranslated RNA molecule; and

(c) recovering said protein or untranslated RNA molecule;

wherein said protein or untranslated RNA molecule is encoded by said RNA molecule.

95. The method of claim 94, wherein the protein is erythropoietin.
96. The method of claim 94, wherein said RNA is packaged into an alphaviral particle.
97. A method for producing an alphaviral particle, said method comprising:
- (a) introducing into a host cell at least one DNA molecule of claim 75 having one or more open reading frames which encode alphaviral structural proteins to produce a recombinant host cell;
 - (b) growing the recombinant host cell under conditions suitable for the production of an alphaviral particle which contains an RNA transcription product of said DNA molecule; and
 - (c) recovering said alphaviral particle.
98. A method for producing a protein, said method comprising:
- (a) infecting a host cell with an alphaviral particle produced by the method of claim 97 to produce a recombinant host cell;
 - (b) growing the recombinant host cell under conditions suitable for the production of said protein; and
 - (c) recovering said protein;
- wherein said protein is encoded by RNA contained in the alphaviral particle.
99. The method of claim 98, wherein said protein is erythropoietin.
100. A method for regulating the expression of a protein or an untranslated RNA molecule, said method comprising:

(a) introducing at least one DNA molecule of claim 75 into a host cell to produce a recombinant host cell;

(b) growing the recombinant host cell under suitable culture conditions; and

(c) changing the temperature of the recombinant host cell culture from:

(i) a permissive temperature to a restrictive temperature, or

(ii) a restrictive temperature to a permissive temperature;

wherein said protein or untranslated RNA molecule is encoded by said DNA molecule.

101. A method for regulating the expression of a protein or an untranslated RNA molecule, said method comprising:

(a) introducing at least one RNA molecule of claim 90 into a host cell to produce a recombinant host cell;

(b) growing the recombinant host cell under suitable culture conditions; and

(c) changing the temperature of the recombinant host cell culture from:

(i) a permissive temperature to a restrictive temperature, or

(ii) a restrictive temperature to a permissive temperature;

wherein said protein or untranslated RNA molecule is encoded by said RNA molecule.

102. An isolated nucleic acid molecule comprising a polynucleotide having the nucleotide sequence of SEQ ID NO:1.

103. A DNA vector system comprising one or more polynucleotides which encode RNA molecules, said RNA molecules comprising:

(a) at least one *cis*-acting sequence element,

(b) a first open reading frame having a nucleotide sequence encoding a non-cytopathic, temperature-sensitive RNA-dependent RNA polymerase, and

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(c) at least one second nucleotide sequence selected from the group consisting of:

(i) a second open reading frame encoding a protein, or portion thereof, wherein said second open reading frame is in a translatable format after one or more RNA-dependent RNA replication events;

(ii) a sequence complementary to all or part of the second open reading frame of (i); and

(iii) a sequence encoding an untranslated RNA molecule, or complement thereof;

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wherein said second nucleotide sequence is operably linked to a promoter which is activated by said non-cytopathic, temperature-sensitive RNA-dependent RNA polymerase.

104. The DNA vector system of claim 103, wherein the RNA-dependent RNA polymerase is of alphaviral origin.

105. The DNA vector system of claim 103 which encodes an RNA-dependent RNA polymerase having replicase activity at 34°C which is at least five fold lower than the replicase activity exhibited at 29°C.

106. The DNA vector system of claim 103, wherein the second open reading frame encodes a cytokine, a lymphokine, a tumor necrosis factor, an interferon, a toxic protein, or a prodrug converting enzyme.

107. The DNA vector system of claim 103, wherein the second open reading frame encodes human erythropoietin or human β -interferon.

108. The DNA vector system of claim 103, wherein the second nucleotide sequence encodes an untranslated RNA molecule selected from the group consisting of an antisense RNA, a tRNA, a rRNA, or a ribozyme.

109. A method of making a recombinant host cell comprising introducing at least one polynucleotide of claim 103 into a host cell.

110. An *in vitro* cell culture comprising a recombinant host cell produced by the method of claim 109.

111. An *in vitro* cell culture comprising a recombinant host cell comprising at least one polynucleotide of claim 103.

112. The cell culture of claim 111, wherein at least one polynucleotide of claim 103 is stably maintained in said recombinant host cell.

113. A composition comprising one or more RNA molecules transcribed from one or more polynucleotides of the vector system of claim 103.

114. An alphaviral particle containing at least one RNA molecule of claim 113.

115. An *in vitro* cell culture comprising a recombinant host cell comprising at least one RNA molecule of claim 113.

116. A method for producing a protein or an untranslated RNA molecule, said method comprising:

(a) introducing at least one DNA molecule of claim 103 into a host cell to produce a recombinant host cell;

(b) growing the recombinant host cell under conditions suitable for the production of said protein or untranslated RNA molecule; and

(c) recovering said protein or untranslated RNA molecule;

wherein said protein or untranslated RNA molecule is encoded by said DNA molecule.

117. A method for producing a protein or an untranslated RNA molecule, said method comprising:

(a) introducing at least one RNA molecule of claim 113 into a host cell to produce a recombinant host cell;

(b) growing the recombinant host cell under conditions suitable for the production of said protein or untranslated RNA molecule; and

(c) recovering said protein or untranslated RNA molecule;

wherein said protein or untranslated RNA molecule is encoded by said RNA molecule.

118. The method of claim 117, wherein the protein is erythropoietin.

119. The method of claim 117, wherein said RNA is packaged into an alphaviral particle.

120. A method for producing an alphaviral particle, said method comprising:

- (a) introducing into a host cell at least DNA molecule of claim 103 having one or more open reading frames which encode alphaviral structural proteins to produce a recombinant host cell;
- (b) growing the recombinant host cell under conditions suitable for the production of an alphaviral particle which contains an RNA transcription product of said DNA molecule; and
- (c) recovering the alphaviral particle.

121. A method for producing a protein, said method comprising:

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- (a) infecting a host cell with an alphaviral particle produced by the method of claim 120 to produce a recombinant host cell;
 - (b) growing the recombinant host cell under conditions suitable for the production of said protein; and
 - (c) recovering said protein;

wherein said protein is encoded by RNA contained in the alphaviral particle.

122. The method of claim 121, wherein said protein is erythropoietin.

123. A method for regulating the expression of a protein or an untranslated RNA, said method comprising:

- (a) introducing at least one DNA molecule of claim 103 into a host cell to produce a recombinant host cell;
- (b) growing the recombinant host cell under suitable culture conditions; and

- (c) changing the temperature of the recombinant host cell culture from:
 - (i) a permissive temperature to a restrictive temperature, or
 - (ii) a restrictive temperature to a permissive temperature;

wherein said protein or untranslated RNA molecule is encoded by said DNA molecule.

124. A method for regulating the expression of a protein or an untranslated RNA molecule, said method comprising:

- (a) introducing at least one RNA molecule of claim 113 into a host cell to produce a recombinant host cell;
- (b) growing the recombinant host cell under suitable culture conditions; and
- (c) changing the temperature of the recombinant host cell culture from:
 - (i) a permissive temperature to a restrictive temperature, or
 - (ii) a restrictive temperature to a permissive temperature;

wherein said protein or untranslated RNA molecule is encoded by said RNA molecule.

125. A composition comprising one or more RNA molecules, said RNA molecules comprising:

- (a) at least one *cis*-acting sequence element,
 - (b) a first open reading frame having a nucleotide sequence encoding a non-cytopathic, temperature-sensitive RNA-dependent RNA polymerase, and
 - (c) at least one second nucleotide sequence selected from the group consisting of:
 - (i) a second open reading frame encoding a protein, or portion thereof,
- wherein said second open reading frame is in a translatable format after one or more RNA-dependent RNA replication events;